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Contribution to Mn bioavailability assessment in characteristic soil types via E-value determinations

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SUMMARY

The paper intends to support the more dependable assessment of bioavailable manganese portion in soils via widening the conventional methods by application of isotope exchange. Seven samples representing three Slovak soil types were investigated. In equilibration process Ca(II) and Sr(II) while for isotope exchange the radioisotope ^{54}Mn in form of $^{54}MnCl_2$ were utilized. The results of E-value determinations showed significant divergences in soil types as well in their different horizons. Both the equilibration with Ca(II) and the equilibration with Sr(II) brought the highest E-values for Calcic Phaenozem followed by sharply lower ones for Haplic Cambisol and negligible E-values for Haplic Arenosol. The comparison of the relevant E-values for horizons A, B and C showed an unambiguous decrease with the soil depth. At the same time for the exchangeable manganese fraction of the total manganese content ($F_{Mn} = Mn_E/Mn_{total}$) a similar dependence was observable. In Calcic Phaenozem, equilibrated with Ca(II), we determined for horizons A, B and C as much as 600.0 mg · kg^{-1} (F_{Mn} = 0.75), 288.9 mg · kg^{-1} (F_{Mn} = 0.41) and 228.9 mg · kg^{-1} (F_{Mn} = 0.38) exchangeable Mn respectively. In the same soil samples the equilibration with Sr(II) resulted in more or less indistinctive changes. The E-values for horizon A of Haplic Arenosol gained by Ca(II) equilibration 3.7 mg · kg^{-1} (F_{Mn} = 0.04) and by Sr(II) equilibration 9.4 mg · kg^{-1} (F_{Mn} = 0.09). The lowest exchangeable manganese fraction was determined in horizons A, B and C of Haplic Cambisol, where the Ca(II) equilibration resulted in 26.9 mg · kg^{-1} (F_{Mn} = 0.07), 10.8 mg · kg^{-1} (F_{Mn} = 0.03) and 8.6 mg · kg^{-1} (F_{Mn} = 0.03) respectively, while the Sr(II) equilibration resulted in a not fundamental but cognisable increase of both the extractable manganese amounts and the relevant Mn_E values.

Keywords: manganese, E-value, equilibration, bioavailable fraction.

Introduction

For assessment of bioavailable amounts of mostly metallic soil constituents – beside conventional methods including a wide spectra of simultaneous and sequential extractions – a number of sophisticated principles have been proposed. Among them the application of radioisotopes (more recently stable isotopes) enabled the development of a promising group of specific analytical methods based on isotopic exchange. The relevant methods are in some extent analogous as the principle of isotope dilution analysis (*Hamon et al.* 2002, *Bakkaus et. al.* 2008, *Gäbler et al.* 2007). Most known from this method group is that one which put to use of isotopic exchange between a sequentially mixed in amount of appropriate isotopic indicator (of negligible chemical concentration) and the relevant elemental constituent in investigated soil sample. The method leads to determination of the isotopically exchangeable soil component – the E-value (*Goldberg* and *Smith* 1984, 1985). The analytical cycle includes a specific sequence of steps. After an effective contact of the soil sample with ions of a chosen alkaline earth metal (mostly Ca^{2+}), e.g. after an adequate equilibration, the isotopic indicator (radioactive or stable) is added to the soil/solution suspension. The contact of the phases is then continued and in chosen time periods the count rate (in case of stable isotopic indication the concentration of the stable indicator) of the solution is measured. After the count rate (the concentration of the exchangeable stable indicator) becomes time constant – the E-value is calculated. Some of the methods proposed for determination of bioavailable fraction of soil constituents make use of the corresponding principle utilizing plants as a specific sort of separation reagents (*Lesný et al.* 2005). Another particular method has been proposed and tested for purpose of some soil component's labile fraction determination, named L-value. The relevant methods represent some biological assessment concerning mainly metallic soil constituents leading to labile (for plants available) fractions of the investigated component. As by determination of E-value both radioactive and stable indicators are applicable. After an adequate labeling of the soil sample with an appropriate isotope the cultivation of chosen plants is accomplished (as a rule from seeds). Then the total soil component as well as its isotopic fraction in plant tissue is determined and the L-value is calculated (*Midwood* 2007). Up to now published papers reveal, that the gained E- and L-values – representing more or less conformable fraction of investigated soil constituents – belong to useful data helping to improve the characterization of soils and to objectification of their fortification modes. Critical valuation of the relevant results implies that in the vast majority the L-values are higher in comparison with E-values. This knowledge is apparently related to the fact, that the changes in soil composition (first and foremost in the rhizosphere), caused by the living organisms, may significantly modify (increase) the bioavailability of the related soil constituents. However, there are also E- and L-value relations of opposite character, namely those, where due to a broad range of complex chemical and biological processes, the labile pool of the investigated soil constituent is of lower concentration in comparison with the exchangeable one (*Tongtavee et al.* 2005).

Manganese in soils is present in a variety of its chemical forms. It stands for one of the major essential element of soils and together with a number of other metallic and nonmetallic

elements, especially Zn, Cu and Se, at the same time also belongs to potentially toxic ones (*Römheld* and *Marschner* 1991). Data concerning the abundance of Mn in the earth's crust differ significantly in the range from 0.053 wt. % to 0.14 wt. %. In upper continental crust the most reliable value of manganese concentration is usually considered to be 0.06 wt. % (*Taylor* and *McLennan* 1985). Nevertheless, manganese is a biogenic element, having a broad range of physiological functions. Its deficiency in human's nutrition can lead to several dysfunctions including the ones of the central nervous system. Manganese plays an important role in tissue and bone production as well as in metabolism of saccharides and lipids (*Crossgrove* and *Zheng* 2004). General information related to recent knowledge concerned to manganese chemical species, their toxicity and their chemical analysis is to found in our previous publication (*Nádaská et al.* 2010).

Materials and methods

Soil samples and their essential properties

Seven soil samples showing a wide range of physical and chemical properties were chosen for this study. The investigated samples were obtained by own sampling and represented three important soil types of Slovakia, namely Calcic Phaenozem (Siltic) (Jaslovské Bohunice), Haplic Arenosol (Borský Mikuláš) and Haplic Cambisol (Dystric) (Sihla). The related sampling sites are shown in *Figure 1.* and the most important characteristics of the studied samples are depicted in *Table 1.*

Figure 1. Sampling sites of investigated soils

Table 1. Important characteristics of studied soil samples

Sampling site	Horizon	Signin text	Co-ordinates	Classification	pH		CEC meq/100 g	$CaCO_3$ (%)
					H_2O	KCl		
Jaslovslé Bohunice	horizon A (0–20 cm)	JB A	E Long: 17° 64' 20'' N Lat: 48° 47' 02''	Calcic Phaenozem	8.30	7.53	18.05	0.7
Jaslovské Bohunice	horizon B (35–45 cm)	JB B	E Long: 17° 64' 20'' N Lat: 48° 47' 02''	Calcic Phaenozem	8.58	7.80	16.61	2.75
Jaslovské Bohunice	horizon C (≥ 50 cm)	JB C	E Long: 17° 64' 20'' N Lat: 48° 47' 02''	Calcic Phaenozem	8.92	8.34	14.45	14.8
Borský Mikuláš	horizon A (0–15 cm)	BM	E Long: 17° 19' 02'' N Lat: 48° 62' 04''	Haplic Arenosol	6.83	5.93	5.95	0.45
Sihla	horizon A (0–15 cm)	SH A	E Long: 19° 65' 28'' N Lat: 48° 65' 41''	Haplic Cambisol	4.6	3.8	10.99	0.5
Sihla	horizon B (15–25 cm)	SH B	E Long: 19° 65' 28'' N Lat: 48° 65' 41''	Haplic Cambisol	4.7	4.0	6.67	0.5
Sihla	horizon C (≥ 50 cm)	SH C	E Long: 19° 65' 28'' NLat: 48° 65' 41''	Haplic Cambisol	4.7	4.2	8.11	0.5

Preparation of samples

The samples were gathered (gained) by self made field sampling. The relevant samples were air dried, the plant residues were removed and after homogenization and sieving (2 mm) the soil samples were stored at a sufficiently dark and dry place.

Table 2. Concentrations of elemental constituents of the investigated soil samples (RFA)

Sample / Element	Total concentration of elements (mg · kg^{-1})						
	JB A	JB B	JB C	BM	SH A	SH B	SH C
As	11	10	7	< 2	6	5	3
Ba	449	436	346	288	810	818	803
Br	8	11	10	< 2	5	4	6
Ca	1000	104000	26000	9000	5000	5000	5000
Cd	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Ce	88	82	68	< 10	58	5	61
Cr	101	98	76	7	23	21	18
Cs	10	4	3	< 3	8	10	5
Cu	24	21	16	< 5	6	8	6
Fe	3000	2900	2300	2000	2100	2100	2200
Ga	13	13	11	< 3	21	24	24
La	53	43	33	< 10	33	32	34
Mg	3000	14000	12000	10000	7000	7000	6000
Mn	800	700	600	100	400	400	300
Nb	16	16	12	< 2	11	11	12
Ni	42	47	31	5	13	9	7
Pb	25	21	17	12	40	40	33
Rb	105	101	74	42	110	114	111
Se	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Sn	3	3	2	< 2	4	5	4
Sr	103	115	171	47	220	229	222
Th	11	10	6	< 3	9	9	12
V	85	80	68	6	52	55	53
W	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Y	34	32	27	4	11	11	11
Zn	75	70	54	12	59	61	54
Zr	428	408	320	51	186	193	203

Determination of elemental soil constituents: Total concentrations of 27 chosen elements including manganese were determined by RFA using the high performance X-ray fluorescence spectrometer X-LAB 2000, SPECTRO, Germany. The results of the relevant determinations are shown in *Table 2*.

E-value determinations

The quantification of isotopically exchangeable manganese content of soils were accomplished by two slightly modified procedures based on *Goldberg* and *Smith* (1984). The applied modifications differed regarding the chosen modes of equilibration. Namely, for displacement of soil manganese in process of inter-elemental exchange two alkaline earth metal cations of the same concentration and volume – Ca(II) in form of $CaCl_2$ and Sr(II) in form of $SrCl_2$ – were chosen. To 2.5 g soil samples placed in tight 120 cm^3 volume PE vessels 50 cm^3 0.05 mol · dm^{-3} of above mentioned reagents (both analytical grade, Sigma Aldrich, Germany) were added. In order to minimize the microbiological activity to all vessels 0.1 cm^3 of chloroform was added as well. For reaching an adequate degree of chemical equilibration the shaking was maintained for 5 days (*Goldberg* and *Smith* 1984, 1985). To achieve sufficient aeration, the vessels were opened daily for several minutes. The equilibration was carried out at 20±2 ^oC and at shaking frequency 150 min^{-1} using the orbital shaker Multi-Shaker PSU20, BIOSAN, Lithuania. After the expected completion of equilibration the manganese concentrations in the related supernatants gained by centrifugation (5 minutes at RCF = 1789 g) were determined by galvanostatic stripping chronopotentiometry using EcaFlow model GLP 150, ISTRAN Ltd., Slovakia. These values were assigned to be the neutral salt extractable Mn concentrations – generally representing an important value of manganese mobility in the investigated soils. The afforecited manganese concentrations – much like as the manganese contents in the remaining solid phases – were considered to be constant for the subsequent isotopic exchange processes. After reaching the estimated quasi equilibrium state an aqueous solution of $^{54}MnCl_2$ of both negligible volume and negligible chemical concentration was added while the shaking was immediately continued. At defined time intervals the shaking was interrupted, 5 cm^3 of the correspondent suspensions were centrifuged and the count rate of 3 cm^3 of the solid-free solutions were measured applying the gamma spectrometric detection assembly composed of the NaI(Tl) well type scintillation detector 76BP76/3 SCIONIX, Netherlands, operated by the data processing software ScintiVision-32, ORTEC, USA. After detection of the count rates, the related aliquots were returned to studied mixtures and the shaking was resumed. This process was repeated until the isotopic exchange equilibrium was observed manifested by a negligible time dependence of the measured count rates. All measurements were accomplished with four replicate samples. The quantities of the isotopically exchangeable Mn in the investigated soil samples (Mn_E) were calculated using equation (1):

$$Mn_E = \frac{\text{amount of Mn in soil extract / soil weight}}{\text{fraction of } {}^{54}\text{Mn in extact}} \qquad (1)$$

Applied radioindicator

The isotopic exchange experiments were accomplished applying ^{54}Mn in the form of $^{54}MnCl_2$ etalon (specific activity 3,563 MBq · g^{-1}, chemical concentration 50 mg · dm^{-3} $MnCl_2$ in 3 g dm^{-3} HCl) obtained from the Czech Institute of Metrology, Prague (Czech Republic).

Results and discussion

In order to carry out the determinations of E-values characterizing the investigated soil samples we accomplished the isotopic exchange experiments described in the previous chapter. *Figure 2.* shows the time dependence of supernatant's count rates using $CaCl_2$ as equilibration solution for neutral (BM) and acidic (SH A, SH B, SH C) samples and *Figure 3.* the same dependence for basic (JB A, JB B, JB C) ones. *Figure 4.* demonstrates the time dependence of supernatant's count rates using the $SrCl_2$-modification of the equilibration procedure for the neutral and acidic samples (BM, SH A, SH B and SH C) while *Figure 5.* describes the analogous dependence for the basic ones (JB A, JB B and JB C). The summarization of our experimental results and the efforts towards their critical assessment may lead to the next generalizations:

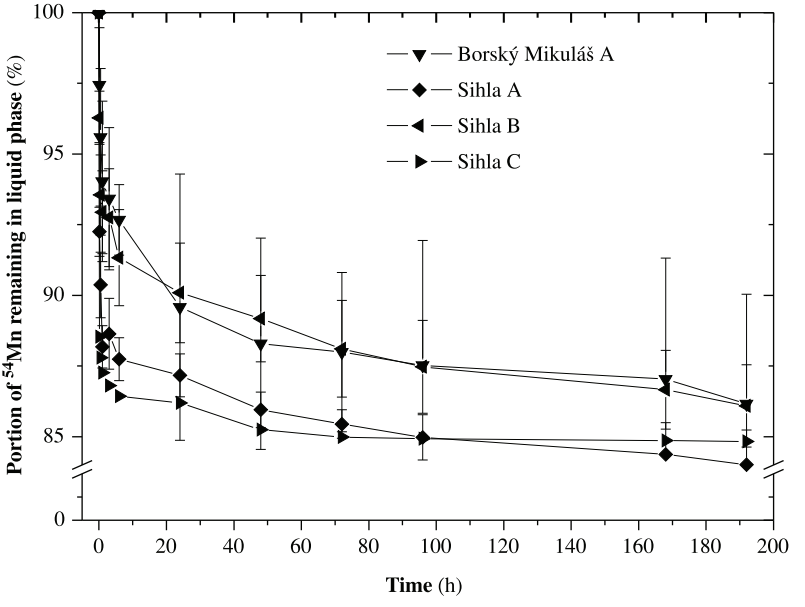

Figure 2. Portion of ^{54}Mn in the liquid phase as a function of contact time for neutral (BM A) and acidic (SH A, SH B, SH C) soil samples applying $CaCl_2$ type of equilibration

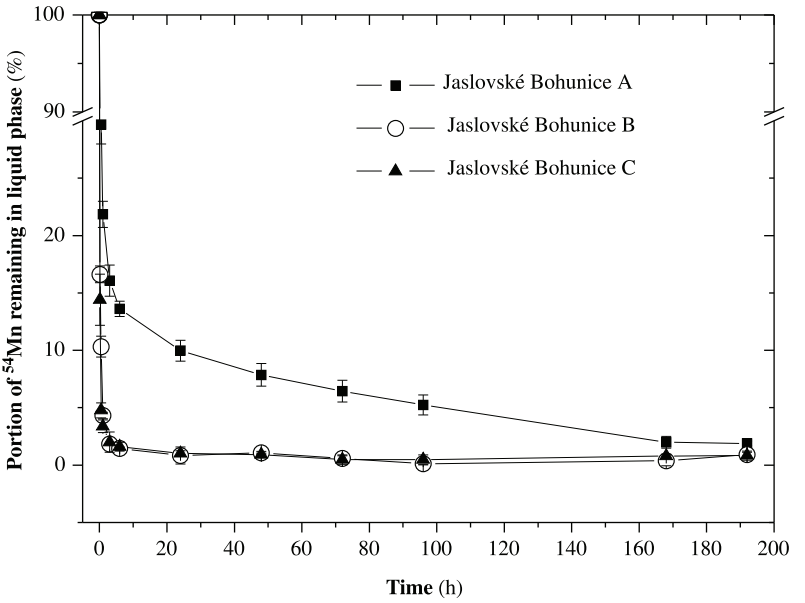

Figure 3. Portion of ^{54}Mn in the liquid phase as a function of contact time for basic soil samples (JB A, JB B, JB C) applying $CaCl_2$ type of equilibration

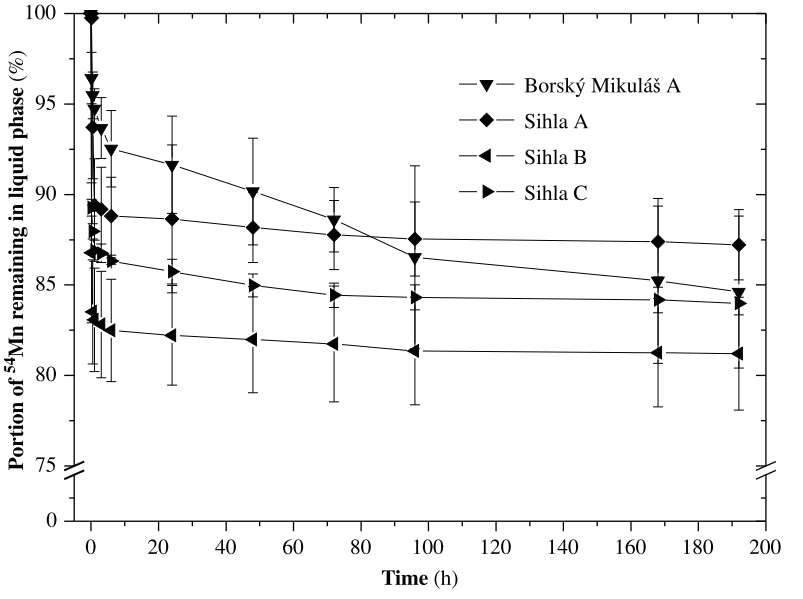

Figure 4. Portion of ^{54}Mn in the liquid phase as a function of contact time for neutral (BM A) and acidic (SH A, SH B, SH C) soil samples applying $SrCl_2$ type of equilibration

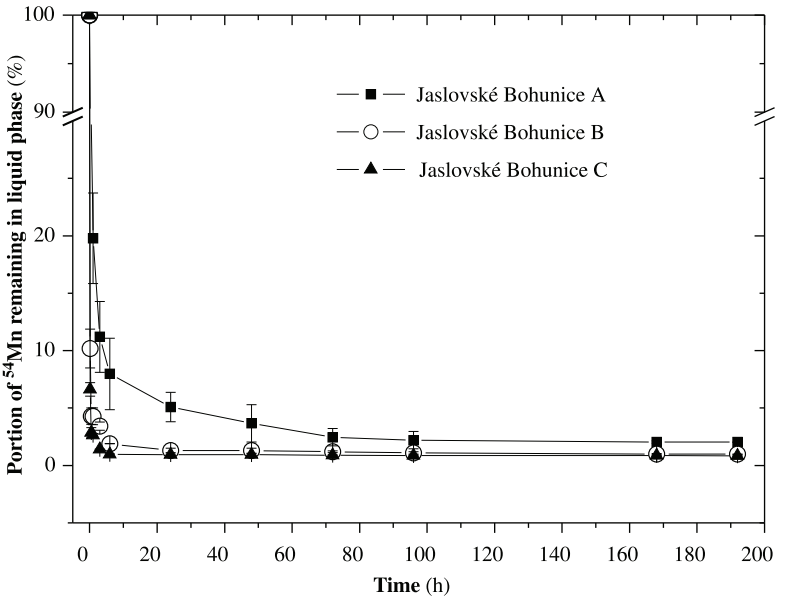

Figure 5. Portion of ^{54}Mn in the liquid phase as a function of contact time for basic soil samples (JB A, JB B, JB C) applying $SrCl_2$ type of equilibration

The quasi equilibrium state, i.e. the quasi stable isotopic composition of liquid/solid phases of the studied suspensions was reached for different soil samples in significantly different times. This judgement is valid for experiments accomplished with both, same sample using different equilibration reagents ($CaCl_2$ and $SrCl_2$) and different soil samples using the same equilibration reagent. Taking in account the equal experimental conditions (temperature, granularity, reagent concentrations, liquid/solid phase ratio) the different contact times required for reaching quasi equilibrium are due to different kinetic parameters of studied isotope exchange.

The contact time required for reaching the apparent isotope exchange equilibrium was the highest for the basic soil sample JB A applying $CaCl_2$ as equilibration solution, namely it made up to 160 hours, whereas the time observed for the same sample applying $SrCl_2$ was as much as 100 hours. Comparing the above stated results with the ones concerning the deeper lying horizons – JB B and JB C – we may ascertain, that applying both of the pertinent equilibration solutions the apparent isotope exchange equilibrium was reached virtually immediately. For the neutral soil sample (BM) the observed isotope exchange contact time varied from ~50 hours applying $CaCl_2$ to ~100 hours applying $SrCl_2$. Although all of the studied horizons of the investigated acidic soil type (SH A, SH B, SH C) applying both of the utilized equilibration solutions – in comparison with horizon A of the basic soil type (JB A) – showed lower contact time requirement for apparent isotope exchange, at the same time they showed significantly higher time demand in comparison with the deeper lying horizons of the basic soil samples (JB B and JB C). However, it is worthwhile to mention that our experimental measurements do not allow reliable quantifications of

these data, which in general shows a higher time requirement for the $CaCl_2$ equilibration (50–60 hours) in comparison with the $SrCl_2$ one (20–30 hours).

A widened series of obtained results applying $CaCl_2$ and $SrCl_2$ respectively are shown in *Table 3.* and *Table 4.* The corresponding tables intend to characterize the investigated soil samples by a specific data set chosen for preferable approximation of bioavailable soil manganese assessment.

Assessing the response of applied equilibration reagents there is an observable increase of extractable manganese content by all investigated samples using $SrCl_2$ in comparison with $CaCl_2$. Our results confirm the generally accepted experience concerning the effect of pH on the manganese extraction, namely the fact that more acidic soils show higher alkali earth metal extractable manganese portions. At the same time we ascertained an inverse dependence regarding the effect of pH on the Mn_E/Mn_{total} ratio. Our measurements led by both equilibration reagents to an unambiguous decrease of the Mn_E/Mn_{total} ratio with increasing soil acidity. Along with the above stated relation the depicted Mn_E/Mn_{total} ratio decreases with the depth of the soil horizon as well as with the decrease of the cation exchange capacity of particular samples.

Table 3. Partial summarization of analytical results related to all investigated soil samples after equilibration with $CaCl_2$

Soil	JB A	JB B	JB C	BM A	SH A	SH B	SH C
Mn_E ($mg \cdot kg^{-1}$)	600.0±75.9	288.9±65.2	228.9±110.9	3.7±0.4	26.9±1.3	10.8±0.5	8.6±0.8
Mn_{total} ($mg \cdot kg^{-1}$)	800.0±64.0	700.0±56.0	600.0±48.0	100.0±8.0	400.0±32.0	400.0±32.0	300.0±24.0
$MnCaCl_2$ ($mg \cdot kg^{-1}$)	11.4±0.8	2.6±0.1	1.9±0.1	3.2±0.3	22.7±1.1	9.4±0.4	7.3±0.7
pH_{H_2O}	8.3	8.6	8.9	6.8	4.6	4.7	4.8
Mn_E/Mn_{CaCl_2}	52.6±7.6	111.1±25.4	120.5±58.7	1.2±0.2	1.2±0.1	1.1±0.1	1.2±0.2
Mn_E/Mn_{total} (%)	75.0±11.2	41.3±9.9	38.2±18.7	3.7±0.5	6.7±0.6	2. ±0.2	2.9±0.4
Mn_{CaCl_2}/Mn_{total} (%)	1.4±0.2	0.4±3.3	0.3±3.0	3.2±0.4	5.7±0.5	2.35±0.2	2.4±0.3
Residual ^{54}Mn portion in liquid phase (%)	1.9±0.2	0.9±0.2	0.8±0.4	86.2±3.9	84.1±1.2	86.1±1.5	84.8±0.51

Table 4. Partial summarization of analytical results related to all investigated soil samples after equilibration with $SrCl_2$

Soil	JB A	JB B	JB C	BM A	SH A	SH B	SH C
Mn_E ($mg \cdot kg^{-1}$)	552.3±62.9	425.0±121.9	225.0±20.5	9.4±0.8	39.7±1.1	28.1±1.5	24.4±0.9
Mn_{total} ($mg \cdot kg^{-1}$)	800.0±64.0	700.0±56.0	600.0±48.0	100.0±8.0	400.0±32.0	400.0±32.0	300.0±24.0
Mn_{SrCl_2} ($mg \cdot kg^{-1}$)	11.6±1.2	5.1±1.4	2.7±0.1	8.0±0.5	34.6±0.5	22.8±0.8	20.5±0.7
pH_{H_2O}	8.3	8.6	8.9	6.8	4.6	4.7	4.8
Mn_E/Mn_{SrCl_2}	47.6±7.3	83.3±33.1	83.3±8.2	0.3±0.1	1.1±0.1	1.2±0.1	1.2±0.1
Mn_E/Mn_{total} (%)	69.0±9.6	60.7±18.1	37.5±4.5	9.4±1.1	9.9±0.8	7.0±0.7	8.1±0.7
Mn_{SrCl_2}/Mn_{total} (%)	1.5±0.2	0.7±0.2	0.5±3.9	8.0±0.8	8.7±0.7	5.7±0.5	6.8±0.6
Residual ^{54}Mn portion in liquid phase (%)	2.1±0.1	1.2±0.1	1.2±0.1	85.2±4.2	87.2±1.9	81.2±3.1	83.9±1.6

Conclusions

For assessment of biologically available soil manganese isotopically exchangeable manganese concentrations were determined in seven soil samples representing three characteristic Slovak soil types. Reliable estimation of bioavailable soil constituents belong to cumbersome and scarcely accomplishable analytical problems. Nevertheless, the relevant data are widely required not only by agronomists and paedologists but in rising extent for needs of rapidly developing technologies belonging to phytoremediation. Among frequently discussed and rather convenient experimental methods simultaneous and sequential extractions belong to the most suitable ones but unfortunately no method fulfilled the demanding expectations up to now. Our results may contribute to handle the relevant group of problems by following summarizations:

- The quantification of Ca(II) and Sr(II) extractable soil manganese according to our results leads to generally negligible differences and does not allow significant objectification of bioavailability.
- While analysing basic soil samples, we found distinguished differences (up to two orders) between Mn_E values and $CaCl_2$ ($SrCl_2$) extractable Mn, the relevant differences found by neutral and acidic soil samples are relatively low (not exceeding units of percents).
- While the increasing carbonate content of the investigated samples led to decrease of their Ca(II) and Sr(II) extractable soil manganese, the effect of soil carbonate content on the relevant E-values is less clear.
- While more acidic soils show higher alkali earth metal extractable manganese portions (Mn_{CaCl_2}/Mn_{total} or Mn_{SrCl_2}/Mn_{total}) in comparison with the neutral and basic ones, we ascertained an inverse dependence regarding the effect of pH on the Mn_E/Mn_{total} ratio.
- The measurements confirmed a significant decrease of the Mn_E/Mn_{total} ratio with increasing soil acidity and its decrease with the depth of the soil horizon.
- The decrease of CEC value of investigated soil samples led to decrease of their Mn_E/Mn_{total} ratio.

E-érték meghatározása néhány talajtípus biológiailag hozzáférhető Mn-tartalmának megismeréséhez

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Összefoglalás

A dolgozat a hagyományos analitikai lehetőségek kibővítésével igyekszik a talajok biológiailag hozzáférhető mangántartalmának megbízhatóbb felméréséhez hozzájárulni. A vizsgálatok izotópcsere módszer alkalmazásával történtek. Három szlovákiai talajtípust képviselő, összesen hét talajminta került vizsgálat alá. A talajokban található mangán extrakciójához (az egyensúlyozáshoz) kalcium-kloridot, illetve stroncium-kloridot, az izotóp cseréhez ^{54}Mn-nal jelölt mangán(II)kloridot alkalmaztunk. Az egyes talajtípusok, úgymint az egyes horizontok általunk meghatározott E-értékei, jelentős különbségeket mutattak. Míg a legmagasabb E-értékeket mindkét extrahálószer alkalmazásánál ($CaCl_2$ és $SrCl_2$) a bázikus Calcic Phaenozem típusú talajnál értük el, a savanyú jellegű Haplic Cambisol jelentősen alacsonyabb és a semleges Haplic Arenosol elhanyagolhatóan alacsony E-értékeket mutattak. Az egyes A-, B- és C-horizontokat képviselő minták E-értékei a mélységgel egyértelműen csökkentek. Az izotóposan cserélhető mangántartalom része ($F_{Mn} = Mn_E/Mn_{total}$) a mélységgel való változása hasonló függést mutatott. A Calcic Phaenozem típusú talaj A-, B-, illetve C-horizontjaiban, kalcium-kloriddal való extrahálást alkalmazva, 600,0 mg·kg^{-1} (F_{Mn} = 0,75), 288,9 mg·kg^{-1} (F_{Mn} = 0,41), illetve 228,9 mg·kg^{-1} (F_{Mn} = 0,38) izotóposan cserélhető mangánt határoztunk meg. Az azonos mintákban stroncium-kloridot mint extrahálószert alkalmazva az E-értékek csak jelentéktelen különbségekhez vezettek. A Haplic Arenosol A-horizontját illető E-érték, kalcium-kloriddal való mangán extrakció után, 3,7 mg · kg^{-1} (F_{Mn} = 0,04) volt, míg az alternatív módon extrahálószerrel, stroncium-kloriddal való mangán extrakció után, ez az érték növekvést mutatott (Mn_E = 9,4 mg · kg^{-1}; F_{Mn} = 0,09). A legalacsonyabb izotóposan cserélhető mangántartalom részt (F_{Mn}) a Haplic Cambisol talajtípus A-, B-, illetve C-horizontoknál értük el. Kalcium-kloridot alkalmazva a következő Mn_E értékeket kaptuk: A-horizonton Mn_E = 26,9 mg · kg^{-1} (F_{Mn} = 0,07), B-horizonton Mn_E = 10,8 mg·kg^{-1} (F_{Mn} = 0,03) és C-horizonton Mn_E = 8,6 mg·kg^{-1} (F_{Mn} = 0,03). Stroncium-kloridot alkalmazva az illető Mn_E-értékek, úgymint a stronciummal extrahálható mangán mennyisége is megfigyelhető mértékű növekvést mutattak.

Kulcsszavak: mangán, E-érték, egyensúlyozás, biológiailag hozzáférhető frakció.

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